

EFFECT OF SOME PLANT EXTRACTS AND ESSENTIAL OILS ON CONTROLLING NECK-ROT DISEASE OF ONION.

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ABSTRACT

Onion plant were attacked by neck rot disease caused by *Botrytis allii* (Munn). The modern trends were directed to study the effect of various natural substance against neck rot disease. The effect of plant extract and essential oil on the incidence of plant disease was studied. Plant extracts of six plant extracts, clove (*Dianthus caryophyllus*), cinnamon (*Cinnamomum zeylamicum*), thyme (*Thymus vulgaris* L.) fenugreek (*Trigonella, fonicum*), amme (*Ammi visnaga*), black pepper (*Piper nigrum*) and three oil essential geranium (*Pelargonium graveolens*), black cumin seeds (*Nigella sativa* L.) and blue gum (*Eucalyptus globulus*).

The six plant extracts and the three oil essential were evaluated as antifungal substance on the mycelial growth and incidence and disease severity of neck rot disease. The antifungal properties of cloves extract was more effective than black pepper then Fenugreek extract on inhibiting mycelial and disease, incidence, while amme, thyme cinnamon showed the lowest effect. On the other hand oil essential of geranium was more effective than blue gum and oil black common seeds on inhibiting mycelial growth and disease incidence.

Keywords: *Botrytis allii*, Plant Extracts and Essential oils.

INTRODUCTION

Onion (*Allium cepa*. L.) is one of the most economical field crops in Egypt. Neck rot disease caused by *Botrytis allii* Munn is one of the most serious diseases which attacks onion plants grown for bulb production and during storage. (El-Helaly *et al.*, 1966, Hussein *et al.*, 1977, Maude *et al.*, 1982 and Farrag, Eman 2005). The modern trends are directed to study the effect of various natural substances against some pathogens causes plant diseases. Medical and aromatic plants as antifungal substances were also documented (Saksena and Tripathi, 1987; Agha, 1992; Zedan *et al.*, 1994; Sivropoulou *et al.*, 1995). Wilson *et al.*, 1997 tested some plant extracts and essential oils for their antifungal activity against *Botrytis allii*. The aim of the present work was to study the effect of some plant extracts and some essential oils on controlling neck rot disease of onion.

MATERIALS AND METHODS

1- Isolation and identification of the causal pathogen:

Samples of diseased onion plants showing neck rot disease were collected from different localities of Assiut Governorate. Isolation procedures was carried out using infected onion bulbs Giza 6 cultivar. Soaking the infected plant parts in 1% sodium hypochlorite solution for 10 minutes followed by complete washing in sterile distilled water was used for surface disinfection of infected sample before plating on PDA medium at 20°C for 5-7 days. Pure culture of the developing fungi was obtained by single spore isolat. Primary identification was carried out according to Owen *et al.*, (1950)

and Barnett (1960) then was confirmed by Assiut University Mycological center (AUMC).

2- Pathogenicity test:

Apparently healthy onion bulbs, free from obvious infection by *B.allii* Giza 6 cultivar, were washed thoroughly with tap water, surface sterilized by dipping in 1% sodium hypochlorite solution for 2-3 minutes. Then rewashed with sterile distilled water and left for arial during at room temperature. Inoculum was prepared by growing the three tested isolates on 100ml Potato Dextrose liqued in 250ml. concical flasks, then incubated at 20°C. After 6 days incubation culture filtrates were decanted and the growing mycelium of each tested isolate was washed using sterile distilled water, suspended in 100ml of distilled water and blended in warning blender for two minutes. Healthy onion bulbs were sprayed with the mycelial suspension 4×10^5 propagulus. Fungal suspension was replaced by distilled water and the bulbs were treated in the same manner as a control treatment. Twenty five of bulbs were put in 30 × 40cm. Jute sacks for each treatment replicated four times. Sacks were kept in rows at room temperature about 32°C for three months. A piece of wet cotton with sterilized distilled water was put in each bag to maintain suitable relative humidity around the bulbs. The percentage of infection were recorded after 3 months and disease index of bulbs were recorded after the end of experiment by using scale of oto 4 was used by AICRIP (1968).

3- Plant extract preparation:

Plant extracts were prepared from the indicated parts of the following 6 plant species collected from Assuit Government. Buds of clove, stem of cinnamom, thyme (seeds), Amme (seeds) and black pepper (seeds) and fenugreek (seeds).

According to natural medicine these parts of the tested plant were known to have antifungal and or other dermal qualities (Krispel 1986).

Samples were extracted with steril distilled water by autoclaving at 121°C for 10 min. The concentration of dried material was 10% (w/v). The filtrate was used as test extract as described by Maoz and Neeman (1998).

4- Essential oils extracts:

Three oils of geranium, blue gum and black cumin at the rat of 1.5ml/L were prepared by method described by Baraka *et al.*, (2003).

Effect of some plant extracts on the linear growth of *B.allii* in vitro.

The concentration 10% of clove, black pepper, fenugreek and thymes extracts was prepared by adding suitable amount of sterilized distilled water to the crude extracts of plant (w/v). One ml of each tested plant extract were added to 9ml PDA medium in Petri dishes. Inoculation was done with fungal discs, 5mm in diameter obtained from *B.allii* 7 days old culture. Three replicates were used for each tested concentration.

Another group of PDA plates free from plant extracts, inoculated with the fungus as control. All plates were incubated at 20°C for 7 days. Linear growth was recorded. The obtained data were statistically analyzed, according to Snedecor and Cochracn, (1967).

Effect of three essential oils on the Linear growth of *B.allii* in vitro.

The concentrations 1.5ml/Liter of gernium, black cumin seeds and blue gum essential oils were prepared by adding suitable amount 0.015 of each tested concentration to 10ml PDA medium in Petri dishes.

Inoculation was done with fungal discs, (5mm) in diameter obtained from *B.allii* 7 days old culture. Three replicates were used for each tested concentration. Another group of PDA plates free from essential oils inoculated with the fungus as control. All plates were incubated at 20°C for 7 days. Linear growth was recorded. The percentages of reduction in the mycelial growth were calculated. The obtained data were statistically analyzed, according to Snedecor and Cochran, (1967).

Effect of plant extracts and essential oil on the disease incidence of neck rot disease during storage:

This experiment was carried out in 2004 and 2005. Two isolates of were used *Botrytis allii*. Healthy onion bulbs were sprayed with the mycelial suspension 4×10^5 propagulus/ml and were soaked in plant extracts all essential oils with the same concentration used in *vitro* for 5 minutes. The previously treated bulbs were kept in 30 x 40cm. Jute sacks each containing 25 bulbs and a piece of wetted cotton to maintain suitable humidity around bulbs. Then kept at room temp. (about 32°). Four replicates were used for each treatment a healthy onion bulbs were sprayed with the fungal suspension only used as control. After 90 days from inoculation, percent of infection and disease index of bulbs were recorded after the end of experiment by using scale of 0 to 4 was used by A.I.C.R.I.P. (1968).

Statistical analysis:

The collected data were statistically analyzed using two factor Completely Randomized Block Design. Treatments were compared at 0.05 and 0.01 level of probability L.S.D. (Fisher 1984).

Table (1): Scientific English, and Arabic names of the source used plant extract, Essential oils and their natural component:

Scientific plant extracts and essential oils	English name	Natural component	Arabic name
<i>Dianthus caryophyllus</i>	clove	Eugenol	قرنفل
<i>Cinnamomum zylanicum</i>	cinnamom	Cuminaldehyde	قرفة
<i>Thymus vulgaris, L.</i>	thyme	Thymol and carvacrol	زعتر
<i>Tigonella fonicum</i>	fenugreek	Tergeneol and Colein	حلبة
<i>Ammi visnagal</i>	Khella amme	Khilyen and Glycocid	خلة
<i>Piper nigrum</i>	black pepper	Pippren Kaloyed	الفلفل الأسود
<i>Pelargonium graveolens</i>	geranium	Geraniol	العتبر
<i>Nigella sativa, L.</i>	black cumin seeds	Bachlon	حبة البركة
<i>Eucalyptus globulus</i>	Blue gum camphor	Cineole Thynoleycalptus	الكافور

RESULTS AND DISCUSSION

Data presented in Table (2) indicated that all isolates of *Botrytis allii* causes neck rot disease of onion during storage. Data also show that isolate (No.3) was the highly pathogenic followed by isolate (No.2) then isolate (No.3).

These results were in agreement with the finding of El-helaly *et al.*, 1966 and Hussein *et al.*, 1997 Farrag, Eman 2005.

Table (2): Pathogenicity test of *Botrytis allii* isolates causal pathogen of neck rot of onion.

Isolates	Disease incidence %	Disease severity %
I ₁	40	32.5
I ₂	46	37.37
I ₃	54	42.25
Control	0	0
L.S.D. 5%	2.1328	3.5941
1%	3.0640	5.1634

Data in Table (3) indicate that all plant extracts inhibited linear growth of *Botrytis allii* *in vitro*. compared with control untreated with plant extract. Clove extract was the most active plant extract in inhibiting linear growth of *Botrytis allii* *in vitro* followed by, black pepper, feunogrec; amme, cinnamom and thyme subsequently.

Table (3): Effect of plant extracts on the linear growth of *Botrytis allii* *in vitro*.

Plant extracts	Isolates	Linear growth (mm)
Cloves	I ₁	27.5
	I ₂	20
Mean		23.75
Cinnamom	I ₁	84.1
	I ₂	82
Mean		83.05
Thyme	I ₁	85
	I ₂	85
Mean		85
Feunogreek	I ₁	67.5
	I ₂	60
Mean		63.75
Amme	I ₁	76
	I ₂	70
Mean		73
Black pepper	I ₁	57.5
	I ₂	51.6
Mean		54.55
Control	I ₁	89
	I ₂	89
Mean		89
L.S.D. 5%		I 4.430
		P 8.288
		IXP 11.720
1%		I 5.976
		P 11.180
		IXP 15.810

I Isolates P Plant extracts. IXP Isolates interaction x Plant extracts.

The present work is similar to that reported by Nadia and Nasif 2002. He found that clove and *eucalyptus* oils strongly reduced the mycelial growth of the *Macrophomina phaseolina*, *Fusarium solani*, *Rhizoctonia solani* and *Fusarium semitectum*. Dawood *et al* 2003 found that the extract of (*Nigella sativa* seeds) reduce the dry weight of the *Alternaria alternata*, *Botrytis cinerea*, *Fusarium oxysporum* *Fusarium solani*, *Rhizoctonia solani*, *Sclerotinia sativa*, *Sclerotinia bataticola* and *Sclerotium rofsii*.

Data in Table (4) indicate that all essential oils inhibited linear growth of *Botrytis allii* *in vitro* when compared with the control. The most reduction was obtained when geranium was used followed by blue gum then black cumin seeds.

Theses result are agree with Singh and Gupta (1992) and Soltan (1998) essential oils have been recognized as having good fungitoxic Pattnaik *et al.*, (1996) they found that *eucalyptus* and peppermint oils were effective against *Fusarium. solani*, *Fusarium. oxysporum* and *M. phaseolina* *in vitro*.

The three essential oil was more effective in isolate 1 than isolate2.

The tested medical and aromatic plants extracts could suppress some plant pathogenic microorganisms due to their activities which include mycelial growth, reduce.

Table (4): Effect of essential oils on the linear growth of *Botrytis allii* *in vitro*.

Essential oils	Isolates	Linear growth (mm)
Geranium	I ₁	50
	I ₂	45
Mean		47.5
Black cumin seeds	I ₁	55.8
	I ₂	51.3
Mean		53.55
Blue gum	I ₁	53
	I ₂	48.3
Mean		50.65
Control	I ₁	89
	I ₂	89
Mean		89
L.S.D. 5%		I 1.797
		O 2.542
		IXO 3.595
1%		I 2.479
		O 3.502
		IXO 4.953

I Isolates. O Essential oil. IXO Isolates interaction x Essential oil.

Formation and morphological malformation However, these antimicrobial effects depends on many factors such as chemical structure methods of determination, concentration, plant species, plant age and other biotic (Omar 2000).

Data presented in Table (5) indicated that the tested plant extracts significantly reduced the effect of the two testing isolates developing of onion neck root disease after 90 days of storage when compared with the control. Plant extracts clove was the most effective treatment in controlling disease incidence and severity through the two tested growing seasons followed by black pepper, fenugreek and thyme respectively.

Table (5): Effect of plant extracts on disease incidence and severity of onion neck-rot after 90 days storage through the growing seasons 2004 and 2005.

Plant extracts	Isolates	Season 2004		Season 2005	
		Disease incidence	Disease severity	Disease incidence	Disease severity
Clove	I ₁	9	8.25	11	10.25
	I ₂	16	14	18	14.5
Mean		12.5	11.12	14.5	12.37
Thyme	I ₁	12	10.75	14	13
	I ₂	21	17.75	24	20.25
Mean		16.5	14.25	19	16.62
Feunogreek	I ₁	11	10	13	12
	I ₂	20	16.5	23	19
Mean		15.5	13.25	18	15.5
Black pepper	I ₁	10	9.5	12	11.25
	I ₂	19	15.5	22	18.5
Mean		14.5	12.5	17	14.87
Control	I ₁	40	32.5	42	35.25
	I ₂	50	39.5	54	42.25
Mean		45	36	48	38.75
L.S.D. 5%		I 1.827 P 2.885 IXP 4.085	I 1.963 P 3.104 IXP 4.390	I 2.370 P 3.747 IXP 5.299	I 2.456 P 3.883 IXP 5.491
L.S.D. 1%		I 2.460 P 3.889 IXP 5.500	I 2.644 P 4.180 IXP 5.912	I 3.191 P 5.046 IXP 7.136	I 3.307 P 5.22 IXP 7.394

I Isolates. P Plant extracts. IXP I Isolates interaction x Plant extracts.

Role in the ability of black pepper, blue gum in control of *Botrytis allii* may be attributed to the presence of sterols α triterpens, phenolic, glycosides, alkaloids and carbohydrates α glycosides, alkaloides and carbohydrates α glycosides, anthraquinone, glycosides, tannins, saponin, flavonoids, cardiac, glycosides (Abd El-All, *et al.*, 2003). Effect of thyme extracts may be attributed to the antifungal activity of the natural components, thymoll Muller-Riebau *et al.*, 1995 gallic acid (Cowan, 1999) and phenolic alcohol, polyphenols and flavones (Vokou *et al.*, 1984). Hassanein and El dokschi, 1997 reported that thyme, pepper, ment and caraway oils showed high antimicrobial activity against *Agrobacterium tumefaciens*, *Pseudomonas solanacearum* and *Erwinia carotovora* in *vitro* and in *vivo*. Medicinal plant

treatments. Akhtar and Mahmood 1996 and Amin, 1999). Finally, it could be of great impact on the future of biotic or and organic farming approach in Egypt treated with some ground plant parts (aromatic and medicinal plants) in order to have clean, save, low cost and toxic free agricultural commodities. It could be concluded that use of natural plant extracts to control fungal plant disease may be extended in the future, instead of fungicides which destroy the material equilibrium and cause sever environmental pollution.

Data presented in Table (6) indicated that natural essential oils tested significantly reduced the development of neck rot disease of onion during the two tested seasons when compared with the control in case of the two tested isolates of *Botrytis allii*. Geranium showed the most effective treatment in controlling neck rot (disease incidence and severity) through 2004, 2005 seasons followed by blue gum and Black cumin seeds. The obtained data are in agreement with those of Singh and Gupta (1992) and Soltan (1998). The tested essential oils extracts may contain a variety of substances such as a fixed or volatile oils, tannins, glucoside, alkaloids, flavonoids and saponins while could suppress some plant pathogenic microorganisms. medical plant aromatic possess fungicidal activity and may have a potential for the control of post harvest diseases (Prasad and Stadel backer, (1974) and Singh *et al.*, (1980). It is also agree with the obtained data also are in agreement with Baraka *et al.*, 2003 theses which mentioned that majoram (Geranium) at the rate 1.5ml/L was the most effective treatment in controlling fruit rots followed by cinnamon and camphor (Blue gum).

Table (6): Effect of essential oils on disease incidence and severity of neck rot disease of onion 90 days, after storage under growing seasons 2004 and 2005.

Essential oil	Isolates	Season 2004		Season 2005	
		Disease incidence	Disease severity	Disease incidence	Disease severity
Geranium	I ₁	11	8.75	13	10.5
	I ₂	14	12	16	13.7
Mean		12.5	10.37	14.5	12.1
Black cumin seeds	I ₁	15	13.5	18	17
	I ₂	20	19	22	18.5
Mean		17.5	16.25	20	17.75
Blue gum	I ₁	13	9	15	13.25
	I ₂	15	13	18	16.5
Mean		14	11	16.5	14.87
Control	I ₁	40	32.5	42	32.25
	I ₂	50	39.5	54	42.25
Mean		45	36	48	37.25
L.S.D. 5%		I 2.979	I 3.055	I 3.181	I 1.905
		O 4.213	O 4.321	O 4.498	O 2.698
		IXO 5.958	IXO 6.110	IXO 6.361	IXO 5.170
L.S.D. 1%		I 4.037	I 4.140	I 4.310	I 2.585
		O 5.709	O 5.855	O 6.096	O 3.656
		IXO 8.074	IXO 8.251	IXO 8.621	IXO 5.170

I Isolates. O. Oil essential IXO Isolates interaction x Oil essential.

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تأثير زيوت ومستخلصات بعض النباتات فى مقاومة فطر البوتريتس
المسبب لمرض عفن الرقبة فى البصل.

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تم دراسة تأثير المستخلص المائى لكل من القرنفل والقرفة والزعتر والحلبة والخلة والفلفل الأسود وزيوت نباتات العتر وحبّة البركة والكافور على النمو الميسليومى للمسبب المرضى بوتريتس ألياي، نسبة الإصابة وشدة الإصابة بالمرض. وقد وجد من النتائج المتحصل عليها إن المستخلص المائى لنبات القرنفل كان ذو تأثير أكثر فعالية من المستخلص المائى للفلفل الأسود يليه مستخلص الحلبة فى تثبيط النمو الميسليومى للفطر بينما كان تأثير المستخلص المائى للخلة والقرفة والزعتر أقل فعالية فى تثبيط النمو الميسليومى للفطر. كما أظهرت الدراسة أن زيت العتر كان أكثر فعالية فى تثبيط النمو الميسليومى للفطر يليه الكافور ثم زيت حبة البركة المسبب للمرض. وقد وجد أن النتائج المتحصل عليها فى المعمل أعطت نتائج مماثلة لها فى المخزن. قد أظهر المستخلص المائى لنبات القرنفل تأثير أفضل من المستخلص المائى للفلفل الأسود ثم الحلبة فى تقليل نسبة الإصابة وشدة الإصابة بالمرض فى حين أن زيت العتر كان أفضل من زيت الكافور ثم زيت حبة البركة فى خفض نسبة الإصابة وكذلك شدة الإصابة بالمرض.